## Medical News & Perspectives

# First Gene Therapy for Inherited Hypercholesterolemia a Partial Success

NEW GENES delivered to her liver have significantly lowered the life-threatening cholesterol levels of a 29-year-old Canadian woman. The woman has severe familial hypercholesterolemia, the rare disease in which the liver lacks the receptor for low-density lipoprotein (LDL) and is therefore incapable of clearing the "bad" form of cholesterol from her blood.

The researchers who performed the procedure are predicting that a less invasive gene therapy protocol may be a viable approach to the milder form of the disease if further trials continue to demonstrate safety and efficacy. The milder form affects one in 500 people, placing them at high risk for heart attacks in their 40s and 50s. People with other enzymatic and metabolic disorders may also make good candidates for this new treatment approach, particularly lipid storage diseases and disorders in which the body lacks the enzymes that break down sugars into usable forms.

### Repairing the Cell, Ex Vivo

Last June, investigators at the University of Michigan Medical Center, Ann Arbor, surgically removed 10% of the patient's liver. In a laboratory, they then cultured her hepatocytes and infected these cells with a modified retrovirus that contains an LDL receptor gene. The retrovirus inserts the gene, along with the rest of its genetic material, at a random site in the hepatocyte chromosome.

After 3 days, the investigators infused these repaired cells into the portal vein through a catheter inserted at the time of surgery. The cells are expected to implant and begin expressing the LDL receptor.

Before the procedure, the woman's LDL cholesterol ranged from 12.93 to 16.81 mmol/L (500 to 650 mg/dL). Since the gene therapy, her LDL cholesterol concentration has fluctuated between 20% and 40% below preoperation levels without the assistance of cholesterol-lowering drugs, reported principal investigator James Wilson, MD, PhD, at the December meeting of the Recombinant DNA Advisory Committee of the National Institutes of Health (NIH), Bethesda, Md. Wilson is chief of the division of molecular medicine and genetics, associate professor of internal medicine and of biological chemistry, and an associate investigator with the Howard Hughes Medical Institute, University of Michigan.

A recent liver biopsy showed that the gene was operating in the woman's liver. Wilson and his colleagues now hope to further reduce the woman's cholesterol with cholesterol-lowering drugs, which they began administering in late November. The patient has been responding thus far, Wilson says. She had been unresponsive to these drugs before the gene therapy because the drugs work by stimulating the activity of the LDL receptors—which she lacked.

It remains to be seen how long the gene therapy will work. In theory, it could last for the life of the patient because of the longevity of hepatocytes and because the retrovirus inserts DNA permanently into the genome of the host cell. But this is the first patient to undergo this therapy, and there is still much to be learned.

It is important to note that the woman has experienced no serious complications as a result of the therapy, Wilson says.

The procedure is the second gene therapy protocol for a genetic disease to gain the NIH advisory committee's approval. It is also one of the first two federally approved gene therapy trials to be conducted outside the NIH campus. (The second, also to be conducted at the University of Michigan, involves injecting DNA that encodes a histocompatibility antigen directly into melanoma tumors in an effort to tag these cells for destruction by the immune system.)

### Four More Patients Approved

The NIH committee originally approved three patients for the cholesterol trial, and last December gave its blessing for two more based on the first patient's promising response. Investigators plan to include among these patients children as young as 7 years of age in hopes of preventing the cholesterol buildup in the first place.

The first patient, a woman from outside Quebec City, Quebec, is one of the oldest surviving patients with severe familial hypercholesterolemia. The severe form of the disease afflicts only one of 1 million people in the United States but is 20 times more prevalent among Canadians of French ancestry. The condition develops when an individual inherits two defective copies of the LDL receptor gene. With no means to sop up LDL cholesterol from the blood, the cholesterol rapidly escalates to life-threatening levels. Patients with this condition frequently develop xanthomas, or fatty deposits, around their elbows and knees. One third of the children with the condition suffer heart attacks and angina by the time they are 10 years of age.

This particular patient had suffered a heart attack at the age of 16 years, and underwent a coronary artery bypass graft procedure at the age of 24 years. The bypass graft was beginning to fail. Both of her brothers had died of myocardial infarctions, one at the age of 20 years and the other at 26 years.

Interestingly, says Wilson, both of this patient's affected genes had an identical mutation, which is unusual. Researchers have traced this mutation, and several others in the LDL receptor gene, back to nine families in 16th-century France. Although some mutations cause a complete obliteration of LDL receptor activity, resulting in cholesterol levels in excess of 31.03 mmol/L (1200 mg/dL), this woman's mutation allowed for some residual activity.

### 'Mild' Form Still Lethal

About one in 500 adults in the United States, and one in 80 Quebécois, have the milder form of the disease, caused by the inheritance of one defective LDL receptor gene. These individuals have cholesterol levels in the 9.05 mmol/L (350 mg/dL) range, and are at high risk of having heart attacks in their 40s and 50s. Researchers estimate that 5% of heart attack patients under the age of 45 years have this less severe form of familial hypercholesterolemia.

"In the long term, if gene therapy can be found to be safe and effective in the most severe cases, it then becomes applicable to the heterozygote, which would be a very large number of people," says Francis Collins, MD, PhD, director of

the Human Genome Cer[illegible] the University of Michigan Medical Center. He is also professor of internal medicine and of human genetics, and is a Howard Hughes Medical Institute investigator at the university.

Wilson predicts that other gene delivery systems will eventually replace ones that are considered state of the art today. The current model, in which the gene is delivered ex vivo to the tissue, exposes the patient to the risks of major abdominal surgery and has other inherent limitations. Only a small portion of the liver can be removed safely and, unlike other models based on immune cells, hepatocytes do not divide more than once or twice in tissue culture.

The procedure, at this point, is a long way from providing a cure. "I suspect that we have functionally corrected somewhere around 3% to 4% of the liver cells," Wilson says. But even a small change is significant. "If we can correct 5% of the cells, we will change the condition from the severe to the mild form; over 15%, the patient can have a normal life. We are aiming for 25%, but of course 100% would be ideal."

### Other Promising Strategies

Wilson and his team are working on two innovative strategies that may improve expression levels. The first strategy, involving the adenovirus, was discovered serendipitously. Wilson, Collins, and their colleagues have been working with this virus as a potential vehicle to deliver genes to the lungs of patients suffering from cystic fibrosis.

Although the adenovirus normally infects the body via the upper respiratory tract, the investigators on a whim injected the virus into the bloodstream of a rabbit. Much to their surprise, histologic examination revealed that 98% of the injected virus infected the rabbit's liver, and furthermore, that the infected cells expressed a marker protein carried by the virus. By upping the dosage of virus, the investigators were able to infect 100% of the liver cells after just one injection, in both rabbits and primates.

"Who would have expected that?" asks Wilson, still astounded by the discovery. "Just by coincidence, this virus happens to be hepatotropic, even though we are normally not exposed to it in our circulation."

The investigators have evaluated expression of the marker protein up to 10 days after injection, and it holds steady during that period. They have now created an adenovirus that expresses the LDL receptor and are beginning to test it in animals. "Hopefully, we will take advantage of this technology and begin to deliver the appropriate dose of the functional gene," Wilson says.

The second strategy doesn't involve any virus. Instead, investigators have engineered a "pseudovirus"—submicroscopic spheres made up of thousands of copies of the LDL receptor gene coated with proteins that bind to liver cells.

Researchers injected this "gene drug," as Wilson calls it, into a strain of rabbits that are deficient in the LDL receptor. The rabbits' cholesterol levels dropped immediately, but then rose to preinjection levels after 7 days (*J Biol Chem* 1992;267:963-967).

### Reconfiguring Protein Coat

Wilson notes that this approach is still longer lasting than most pharmaceuticals, but that he would still like to improve the therapy's longevity. The DNA protein complex, as it functions currently, appears to dump its genetic material into the cellular cytoplasm, where it is vulnerable to degradation. The researchers are now working on reconfiguring the protein coat so that it homes in on the nucleus. Wilson says this type of noninvasive gene delivery, if successful, could be used to treat a wide spectrum of human diseases.

"The important lesson that I have learned in gene therapy is this," Wilson says: "Don't be constrained by what you perceive as traditional biases and dogma. Just get out there and start trying a lot of things."

Wilson has also received permission to attempt to deliver genes to the lungs of 12 patients with cystic fibrosis using an adenovirus delivery system. This March, he will move his entire operation to the University of Pennsylvania Medical Center, Philadelphia, where he will direct a new Institute for Human Gene Therapy. The 12 patients with cystic fibrosis and two patients with familial hypercholesterolemia will undergo gene therapy there.—by Teri Randall

# Researchers Try New Definitions, New Therapies in Effort to Solve Growing Problem of Sepsis

PRELIMINARY but promising results of a phase 2 trial of a therapy for sepsis were reported at the 32nd annual Interscience Conference on Antimicrobial Agents and Chemotherapy, held in Anaheim, Calif.

Although the nonblinded study involved a small number of patients, the researchers are encouraged by the strong dose response seen and are looking forward to analyzing the results of a larger, multicenter phase 3 trial just completed.

At the conference, which was sponsored by the American Society for Microbiology, Steven M. Opal, MD, associate professor of medicine, Brown University School of Medicine, Providence, RI, reported the results of the phase 2 trial of a recombinant interleukin 1 receptor antagonist (IL-1ra). The nonblinded, prospective trial involved 99 patients with severe sepsis or septic shock who were randomly divided into a placebo group and three treatment groups, each with approximately 25 patients.

The day before he presented his data at the conference, Opal and two other experts on sepsis took part in a roundtable discussion for medical journalists, which was sponsored by the pharmaceutical company Synergen, Inc, Boulder, Co, manufacturer of the investigational anti–interleukin 1 agent (Antril).

### Blocks Inflammation Mediator

A naturally occurring human protein, IL-1ra inhibits the action of interleukin 1, a potent mediator of inflammation that is produced by immune cells in response to infection or injury.

According to Opal, a strong dose-related survival benefit was seen in patients treated with IL-1ra compared with patients who received placebo. While 56% of patients in the placebo group were alive 28 days after the beginning of treatment, 68% of patients who received a "low dose" (17 mg/h) of IL-1ra, 75% of patients who received a "medium dose" (67 mg/h), and 84% of those who received a "high dose" (133 mg/h) were alive.

The investigational drug was infused over a 3-day period. No overt clinical or laboratory evidence of toxicity or allergic reactions was observed, and no antibodies for IL-1ra were detected, Opal reports.

Although the trial involved a small number of patients, Opal says he and his colleagues found a consistent dose response that suggests the therapy had a